

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
22 April 2004 (22.04.2004)

PCT

(10) International Publication Number
WO 2004/033649 A3

(51) International Patent Classification⁷: C12Q 1/68

(21) International Application Number:
PCT/US2003/031874

(22) International Filing Date: 7 October 2003 (07.10.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/417,009 7 October 2002 (07.10.2002) US

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(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

- with international search report
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments

(88) Date of publication of the international search report:
26 August 2004

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: HIGH THROUGHPUT MULTIPLEX DNA SEQUENCE AMPLIFICATIONS

(57) Abstract: The present invention provides methods of designing PCR primers that allow the efficient and simultaneous amplification of a large number of different desired DNA fragments in a single multiplex PCR and minimize the formation of nonspecific extensions of undesired DNA fragments. The present invention allows a multiplex PCR to use at least 50 pairs of primers and produce at least 50 DNA fragments of interest. The present invention significantly broadens the application of multiplex PCR in the identification of multiple genes related to multifactorial diseases, the genome-scale detection of genetic alterations, the studies in large-scale pharmacogenetic reactions, the genotyping genetic polymorphism in a large population, the gene expression profiling in various samples, and high throughput genotyping technologies.

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US03/31874

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C12Q 1/68

US CL : 435/6

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
west 2.0, caplus, medline, embase, biotechds, biosis, scisearch

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|---|-----------------------|
| A | REMM et al. Primer Design for large-scale multiplex PCR and arrayed primer extension (APEX). PCR Technology, January 2004 (01.2004), pages 131-140, publisher CRC Press LLC, Boca Raton, Florida, entire reference. | 1-12 |
| A | LIN et al. Multiplex Genotype determination at a large number of gene loci. March 1996 (03.1996), Vol. 93, pages 2582-2587, see entire reference | 1-12 |
| A | GORELENKOV et al. Set of Novel tools for PCR primer design. December 2001 (12.2001), Vol. 31, pages 1326-1330, see entire reference. | 1-12 |
| A, E | HEROLD et al. Oligo Design: a computer program for development of probes for oligonucleotide microarray. December 2003 (12.2003), Vol. 35, pages 1216-1221, see entire reference. | 1-12 |



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Date of the actual completion of the international search

22 April 2004 (22.04.2004)

Date of mailing of the international search report

02 JUL 2004

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